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(JP). DAIMON, Kosaku [JP/JP]; c/o Novozymes Jaoan Ltd., Makuhari Techno Garden CB-6, 3, Nakase 1-chome, Seoul, Chiba 261 - 01 (JP).

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(74) Common Representative: NOVOZYMES A/S; Patents, Krogshoejvej 36, DK-2880 Bagsvaerd (DK).

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(71) Applicant (*for all designated States except US*): NOVOZYMES A/S [DK/DK]; Krogshoejvej 36, DK-2880 Bagsvaerd (DK).

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(72) Inventors; and

(75) Inventors/Applicants (*for US only*): UYAMA, Naoto [JP/JP]; c/o Novozymes Japan Ltd., Makuhari Techno Garden CB-6, 3, Nakase 1-chome, Seoul, Chiba 261 - 01

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: REDEPOSITION OR BACKSTAIN INHIBITION DURING STONEWASHING PROCESS

(57) Abstract: During stonewashing of denim fabric or garments, redeposition of blue color often occurs back onto the surface of the denim or other parts like pocket parts. The invention relates to the inhibition of backstaining or redeposition during the stonewashing process by applying a lipolytic enzyme, preferably cutinase, thereby avoiding that the blue colour redeposits on the fabric or garment.

WO 01/92453 A1

REDEPOSITION OR BACKSTAIN INHIBITION DURING STONEWASHING PROCESS**FIELD OF INVENTION**

5 The present invention relates to compositions and methods for reducing or preventing the backstaining of dye on textile materials, especially indigo on denim and specially the backstaining of pocket parts of denim during the stonewashing of denim fabric.

10

BACKGROUND OF THE INVENTION

By stonewashing of denim the usually blue-dyed denim is given a faded or worn appearance with the characteristic white and
15 blue contrast. Stonewashing the denim material is typically carried out in the presence of pumice stone or cellulase or a combination thereof and results in the removal of dye to give areas of lighter colour. The use of cellulase instead of pumice stone has the advantages that it is more environmental friendly,
20 more economical and prevents that the denim is damage because of the rough treatment with the pumice stones. However, the use of cellulase is not without disadvantages.

The dye removed from the denim material after the treatment with cellulase or by a conventional washing process may cause
25 "backstaining" or "redeposition" onto the denim material, e.g. re-colouration of the blue threads and blue coloration of the white threads, resulting in a less contrast between the blue and white threads. In order to remove the dye the denim manufactures are using huge amount of surfactants to make parts white again
30 at a soaping process with heavy washing condition. The heavy washing condition causes colour change or colour-fading problems for finished denim products. Also additional water has to be used in the subsequent soaping process.

The problem of redeposition or backstaining of dye during
35 stonewashing has also been addressed by adding anti-redeposition

chemicals, such as surfactants or other agents into the cellulase wash. Also the use of different cellulases with less specific activity on denim has been tried. WO-A-9407983 describes the use of a cellulase to inhibit the backstaining of
5 denim. WO-A-9429426 and WO-A-9325655 describes backstain inhibition by treatment with a redoposition cellulase composition and added protease as an improvement over the use of redoposition cellulase alone.

Although, these methods aim to solve the problem with the
10 backstaining or redoposition of dye onto the denim material, they may still be improved. Especially, the backstaining or redoposition of dye onto the pocket parts of the denim material pose a problem.

15

SUMMARY OF THE INVENTION

We have developed a process for treating fabric, especially indigo-dyed denim, with a composition comprising a lipolytic
20 enzyme.

This treatment reduces the risk of back-staining (redoposition of dye onto textile) even when less water is used. The enzymatic treatment of released dyestuff will decrease process time as well as the amount of energy and water needed to
25 achieve a satisfactory quality of the textile, and the colour of the wastewater is reduced.

The method of the invention can result in a decreased number of washes, thereby increasing the productivity and decreasing the consumption of water and chemicals, including surfactants.

30 Accordingly, the present invention provides a method for reducing the backstaining of fabric or textile, comprising contacting the fabric or textile with a composition comprising an effective amount of a lipolytic enzyme (EC 3.1.1).

In another aspect, the present invention relates to a
35 stonewashing composition comprising a lipolytic enzyme and a

cellulase.

In a third aspect, the invention relates to the use of the composition for reducing backstaining of fabric or textile.

5

DETAILED DISCLOSURE OF THE INVENTION

Denim that is stonewashed with the addition of an effective amount of added lipolytic enzyme during cellulase treatment
10 shows a reduction in the level of backstaining, especially the backstaining of pocket parts.

The method of the present invention comprises contacting the denim to be enzymatically stonewashed with a composition comprising the lipolytic enzyme in a amount sufficient to reduce
15 backstaining and thus, to decrease the blue-colouring of e.g. the pocket parts.

The amount of added lipolytic enzyme depends upon others on the purity and amount of cellulase used in the stonewashing process, the contact time, the amount of dye removed during
20 stonewashing, the activity of the cellulase, the pH and temperature of the stonewashing process, the formulation of the product and the like.

The composition to be added may further comprise various adjuvants as known to the skilled person, e.g. surfactants.
25 Other materials can also be used with the composition as desired, including stones, fillers, solvents, buffers, pH control agents, enzyme activators, builders, enzyme stabilizers, other anti-deposition agent and the like. The composition may be formulated at a solid product, granular product or as a liquid
30 product.

The lipolytic enzyme may be added to the composition containing the cellulase for use in stonewashing process or added directly to the stonewashing bath or to a subsequent rinse treatment. The lipolytic enzyme may also be added to a
35 composition for washing purposes thereby reducing or inhibiting

the backstaining of removed dye during the washing process.

Fabrics

5 The process of the present invention applies to fabrics in general. In the context of this invention fabrics include fabrics or textiles prepared from man-made fibers, e.g. polyester, nylon, etc., as well as cellulosic fabrics or textiles.

10 The term "cellulosic fabric/textile" indicates any type of fabric, in particular woven fabric, prepared from a cellulose-containing material, containing cellulose or cellulose derivatives, e.g. from wood pulp, and cotton. The main part of the cellulose or cellulose derivatives present on the fabric is
15 normally size with which the yarns, normally warp yarns, have been coated prior to weaving. In the present context, the term "fabric" is also intended to include garments and other types of processed fabrics. Examples of cellulosic fabric is cotton, viscose (rayon); lyocell; all blends of viscose, cotton or
20 lyocell with other fibers such as polyester; viscose/cotton blends, lyocell/cotton blends, viscose/wool blends, lyocell/wool blends, cotton/wool blends; flax (linen), ramie and other fabrics based on cellulose fibers, including all blends of cellulosic fibers with other fibers such as wool,
25 polyamide, acrylic and polyester fibers, e.g. viscose/cotton/polyester blends, wool/cotton/polyester blends, flax/cotton blends etc. The fabric may also include man-made fibers alone such as polyester fibers.

The process of the invention is preferably applied to
30 cellulose-containing fabrics, such as cotton, viscose, rayon, ramie, linen or mixtures thereof, or mixtures of any of these fibers with synthetic fibers. In particular, the fabric may be denim. The fabric may be dyed with vat dyes such as indigo, direct dyes such as Direct Red 185, sulfur dyes such as Sulfur
35 Green 6, or reactive dyes fixed to a binder on the fabric

surface. In a preferred embodiment of the present process, the fabric is indigo-dyed denim, including clothing items manufactured therefrom.

In a most preferred embodiment, the fabric subjected to the process of the invention is made of hydrophobic fibres such as polyamide fibres, e.g. nylon, acrylic fibres, vinylon and polyester fibres. As mentioned above the fabric may be made of mixtures of different fibres. Especially contemplated is polyester or polyester/cotton mixtures, which are the material used for pocket parts of garments, in particular dyed cotton garments or denim jeans.

Enzyme

The enzymatic process of the invention may be accomplished using any carboxylic ester hydrolases, in particular lipolytic enzyme and/or any biopolyester hydrolytic enzyme. Such enzymes are well known and defined in the literature, cf. e.g. *Borgström B and Brockman H L, (Eds.); Lipases; Elsevier Science Publishers B.V., 1984, and Kolattukudy P E; The Biochemistry of Plants, Academic Press Inc., 1980 4 624-631.*

In the context of this invention lipolytic enzymes are classified in E.C. 3.1.1 and include true lipases, esterases, phospholipases, and lyso-phospholipases. More specifically the lipolytic enzyme may be a lipase as classified by EC 3.1.1.3, EC 3.1.1.23 and/or EC 3.1.1.26, an esterase as classified by EC 3.1.1.1, EC 3.1.1.2, EC 3.1.1.6, EC 3.1.1.7, and/or EC 3.1.1.8, a phospholipase as classified by EC 3.1.1.4 and/or EC 3.1.1.32, a lyso-phospholipase as classified by EC 3.1.1.5 and a cutinase as classified in EC 3.1.1.74.

The lipolytic enzyme preferably is of microbial origin, in particular of bacterial, of fungal or of yeast origin.

In a particular embodiment, the lipolytic enzyme used may be derived from a strain of *Absidia*, in particular *Absidia blakesleena* and *Absidia corymbifera*, a strain of *Achromobacter*, in particular *Achromobacter lophagus*, a strain of *Aeromonas*, a

strain of *Alternaria*, in particular *Alternaria brassiciola*, a strain of *Aspergillus*, in particular *Aspergillus niger* and *Aspergillus flavus*, a strain of *Achromobacter*, in particular *Achromobacter iophagus*, a strain of *Aureobasidium*, in particular
5 *Aureobasidium pullulans*, a strain of *Bacillus*, in particular *Bacillus pumilus*, *Bacillus strearothermophilus* and *Bacillus subtilis*, a strain of *Beauveria*, a strain of *Brochothrix*, in particular *Brochothrix thermosohata*, a strain of *Candida*, in particular *Candida cylindracea* (*Candida rugosa*), *Candida*
10 *paralipolytica*, *Candida tsukubaensis*, *Candida auriculariae*, *Candida humicola*, *Cadida foliarum*, *Candida cylindracea* (*Cadida rugosa*) and *Candida antarctica*, a strain of *Chromobacter*, in particular *Chromobacter viscosum*, a strain of *Coprinus*, in particular *Coprinus cinerius*, a strain of *Fusarium*, in
15 particular *Fusarium oxysporum*, *Fusarium solani*, *Fusarium solani pisi*, and *Fusarium roseum culmorum*, a strain of *Geotricum*, in particular *Geotricum penicillatum*, a strain of *Hansenula*, in particular *Hansenula anomala*, a strain of *Humicola*, in particular *Humicola brevispora*, *Humicula lanuginosa*, *Humicola*
20 *brevis* var. *thermoidea*, and *Humicola insolens*, a strain of *Hyphozyma*, a strain of *Lactobacillus*, in particular *Lactobacillus curvatus*, a strain of *Metarhizium*, a strain of *Mucor*, a strain of *Paecilomyces*, a strain of *Penicillium*, in particular *Penicillium cyclopium*, *Penicillium crustosum* and
25 *Penicillium expansum*, a strain of *Pseudomonas* in particular *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *Pseudomonas cepacia* (syn. *Burkholderia cepacia*), *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Pseudomonas maltophilia*, *Pseudomonas mendocina*, *Pseudomonas mephitica* *lipolytica*, *Pseudomonas*
30 *alcaligenes*, *Pseudomonas plantari*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas putida*, *Pseudomonas stutzeri*, and *Pseudomonas wisconsinensis*, a strain of *Rhizoctonia*, in particular *Rhizoctonia solani*, a strain of *Rhizomucor*, in particular *Rhizomucor miehei*, a strain of *Rhizopus*, in
35 particular *Rhizopus japonicus*, *Rhizopus microsporus* and *Rhizopus*

nodosus, a strain of *Rhodosporidium*, in particular *Rhodosporidium toruloides*, a strain of *Rhodotorula*, in particular *Rhodotorula glutinis*, a strain of *Sporobolomyces*, in particular *Sporobolomyces shibatanus*, a strain of *Thermomyces*,
5 in particular *Thermomyces lanuginosus* (formerly *Humicola lanuginosa*), a strain of *Thiarosporella*, in particular *Thiarosporella phaseolina*, a strain of *Trichoderma*, in particular *Trichoderma harzianum*, and *Trichoderma reesei*, and/or a strain of *Verticillium*.

10 In a more preferred embodiment, the lipolytic enzyme used according to the invention is derived from a strain of *Aspergillus*, a strain of *Achromobacter*, a strain of *Bacillus*, a strain of *Candida*, a strain of *Chromobacter*, a strain of *Fusarium*, a strain of *Humicola*, a strain of *Hyphozyma*, a strain
15 of *Pseudomonas*, a strain of *Rhizomucor*, a strain of *Rhizopus*, or a strain of *Thermomyces*.

In a more preferred embodiment, the lipolytic enzyme used according to the invention is derived from a strain of *Bacillus pumilus*, a strain of *Bacillus stearothermophilus* a strain of
20 *Candida cylindracea*, a strain of *Candida antarctica*, in particular *Candida antarctica* Lipase B (obtained as described in WO 88/02775), a strain of *Humicola insolens*, a strain of *Hyphozyma*, a strain of *Pseudomonas cepacia*, or a strain of *Thermomyces lanuginosus*.

25 In the context of this invention biopolyester hydrolytic enzyme include esterases and poly-hydroxyalkanoate depolymerases, in particular poly-3-hydroxyalkanoate depolymerases. In fact an esterase is a lipolytic enzyme as well as a biopolyester hydrolytic enzyme.

30 In a more preferred embodiment, the esterase is a cutinase or a suberinase. Also in the context of this invention, a cutinase is an enzyme capable of degrading cutin, cf. e.g. Lin T S & Kolattukudy P E, J. Bacteriol. 1978 133 (2) 942-951, a suberinase is an enzyme capable of degrading suberin, cf. e.g. ,
35 Kolattukudy P E; Science 1980 208 990-1000, Lin T S &

Kolattukudy P E; Physiol. Plant Pathol. 1980 17 1-15, and The Biochemistry of Plants, Academic Press, 1980 Vol. 4 624-634, and a poly-3-hydroxyalkanoate depolymerase is an enzyme capable of degrading poly-3-hydroxyalkanoate, cf. e.g. Foster et al., FEMS Microbiol. Lett. 1994 118 279-282. Cutinases, for instance, differs from classical lipases in that no measurable activation around the critical micelle concentration (CMC) of the tributyrine substrate is observed. Also, cutinases are considered belonging to a class of serine esterases.

10 The biopolyester hydrolytic enzyme preferably is of microbial origin, in particular of bacterial, of fungal or of yeast origin.

In a preferred embodiment, the biopolyester hydrolytic enzyme is derived from a strain of *Aspergillus*, in particular
15 *Aspergillus oryzae*, a strain of *Alternaria*, in particular *Alternaria brassiciola*, a strain of *Fusarium*, in particular *Fusarium solani*, *Fusarium solani pisi*, *Fusarium roseum culmorum*, or *Fusarium roseum sambucium*, a strain of *Helminthosporium*, in particular *Helminthosporium sativum*, a strain of *Humicola*, in
20 particular *Humicola insolens*, a strain of *Pseudomonas*, in particular *Pseudomonas mendocina*, or *Pseudomonas putida*, a strain of *Rhizoctonia*, in particular *Rhizoctonia solani*, a strain of *Streptomyces*, in particular *Streptomyces scabies*, or a strain of *Ulocladium*, in particular *Ulocladium consortiale*. In a
25 most preferred embodiment the biopolyester hydrolytic enzyme is a cutinase derived from a strain of *Humicola insolens*, in particular the strain *Humicola insolens* DSM 1800.

In another preferred embodiment, the poly-3-hydroxyalkanoate depolymerase is derived from a strain of *Alcaligenes*, in particular *Alcaligenes faecalis*, a strain of *Bacillus*, in particular *Bacillus megaterium*, a strain of *Camomonas*, in particular *Camomonas testosteroni*, a strain of *Penicillium*, in particular *Penicillium funiculosum*, a strain of *Pseudomonas*, in particular *Pseudomonas fluorescens*, *Pseudomonas lemoignei* and *Pseudomonas oleovorans*, or a strain of *Rhodospirillum*, in particular *Rhodospirillum rubrum*.

Specific examples of readily available commercial lipases include Lipolase® (WO 98/35026) Lipolase™ Ultra, Lipozyme®, Palatase®, Novozym® 435, Lecitase® (all available from Novozymes A/S).

Examples of other lipases are Lumafast™, *Ps. mendocian* lipase from Genencor Int. Inc.; Lipomax™, *Ps. pseudoalcaligenes* lipase from Gist Brocades/Genencor Int. Inc.; *Fusarium solani* lipase (cutinase) from Unilever; *Bacillus* sp. lipase from Solvay enzymes. Other lipases are available from other companies.

20

Process conditions

In the case of denim textiles (especially indigo-dyed denim), the process according to the invention can be carried out simultaneously with a treatment with cellulase (and optionally pumice) to create a desired worn look by forming local variations in colour density, as described in American dye stuff reporter, Sept. 90, D. Kochavi, T. Videbæk and D. Cedroni, Optimizing processing conditions in enzymatic stone washing. The process of the invention can also be carried out simultaneously with enzymatic desizing, i.e. removal of starch size by means of an α -amylase. In a further aspect, the process is a conventional washing process, wherein the enzyme of the invention is added to a conventional detergent composition.

The process of the invention may be carried out at

conventional conditions in a washing machine conventionally used for stone-washing, e.g. a washer-extractor. The enzyme of the invention should be added in an effective amount. By the term "effective amount" is meant the amount sufficient to reduce 5 backstaining as compared to the backstaining effect when not applying the enzyme of the invention. Typical conditions are a temperature of 40-60 °C and a pH of 4.5-7.5. However, the process conditions must be chosen according to the characteristics of the enzyme in question. They are generally in 10 the range 20-100°C, pH 4.5-10.5, typically 30-90°C, pH 4.5-7.5 especially 40-60°C, pH 4.5-6.5. Optionally, conventional additives may be used, e.g. a buffer, a surfactant (anionic and/or non-ionic) and/or a polymer (such as PVP, polyacrylate and polyacrylamide).

15

MATERIALS AND METHODS

Enzymes:

20 Cutinase A (Cutinase variant from *Humicola Insolens* according to US 5,827,719).

Cutinase B (Cutinase variant from *Humicola Insolens* according to US 5,827,719).

Denimax® 362S (available from Novozymes A/S).

25 Lipolase® (available from Novozymes A/S).

Lipolase™ Ultra (available from Novozymes A/S).

Cellusoft® L (available from Novozymes A/S)

30 Lipolytic Activity

The lipolytic activity may be determined using tributyrine as substrate. This method is based on the hydrolysis of tributyrine by the enzyme, and the alkali consumption is registered as a function of time.

One Lipase Unit (LU) is defined as the amount of enzyme which, under standard conditions (i.e. at 30.0°C; pH 7.0; with Gum Arabic as emulsifier and tributyrine as substrate) liberates 1 mmol titrable butyric acid per minute (1 KLU = 1000 LU).

A folder AF 95/5 describing this analytical method in more detail is available upon request to Novozymes A/S, Denmark, which folder is hereby included by reference.

10 Cellulytic Activity

The cellulytic activity may be measured in endo-glucanase units (EGU), determined at pH 6.0 with carboxymethyl cellulose (CMC) as substrate.

A substrate solution is prepared, containing 34.0 g/l CMC (Hercules 7 LFD) in 0.1 M phosphate buffer at pH 6.0. The enzyme sample to be analyzed is dissolved in the same buffer. 5 ml substrate solution and 0.15 ml enzyme solution are mixed and transferred to a vibration viscosimeter (e.g. MIVI 3000 from Sofraser, France), thermostated at 40°C for 30 minutes.

One EGU is defined as the amount of enzyme that reduces the viscosity to one half under these conditions. The amount of enzyme sample should be adjusted to provide 0.01-0.02 EGU/ml in the reaction mixture. The arch standard is defined as 880 EGU/g.

The cellulolytic activity may also be determined in endo-cellulase units (ECU) by measuring the ability of the enzyme to reduce the viscosity of a solution of carboxymethyl cellulose (CMC).

The ECU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxy-methylcellulose (CMC). The assay is carried out at 40°C; pH 7.5; 0.1M phosphate buffer; time 30 min; using a relative enzyme standard for reducing the viscosity of the CMC Hercules 7 LFD substrate; enzyme concentration approx. 0.15 ECU/ml. The arch standard is defined

to 8200 ECU/g.

Colour Measurement

A Nippon Denshoku's spectrophotometer (SE 2000), which was
5 in accordance with JIS Z8722, ASTM E308, ASTM E313 and ASTM
D1925, was used according to the manufacturer's instructions
to evaluate the chromaticity using the change in the colour
space coordinates $L^*a^*b^*$ (CIELAB-system), where as usual:

L^* gives the change in white/black on a scale from 0 to 100,
10 and a decrease in L^* means an increase in black colour
(decrease in white colour) and an increase in L^* means an
increase in white colour (decrease in black colour).

a^* gives the change in red/green, and a decrease in a^* means
an increase in green colour (decrease in red colour), and an
15 increase in a^* means an increase in red colour (decrease in
green colour).

b^* gives the change in blue/yellow, and a decrease in b^*
means an increase in blue colour (decrease in yellow colour),
and an increase in b^* means an increase in yellow colour
20 (decrease in blue colour) (Vide WO 96/12846 NOVO).

The Nippon Denshoku's spectrophotometer (SE 2000) was
operated in the $L^*a^*b^*$ colour space. The light source was D65
standard light. The software used for evaluation was ColorMate
Version 4.05. The illumination and light-receiving conditions
25 of this instrument is 0-45° after spectrum method based on JIS
Z-8722 and was calibrated using the white and black tiles.
Each result was an average of 4 measurements. Fabric rinsed
without enzyme and mediator was measured and the coordinates
 $L^*a^*b^*$ were calculated and entered as a reference. The
30 coordinates of the samples were then for each of L^* , a^* , b^*
calculated as the difference of the average of the
measurements on each swatch from the reference value.

The present invention is further illustrated in the
35 following examples, which are not in any way intended to limit

the scope of the invention as claimed.

EXAMPLE 1

5

Comparison of anti-back staining effect between Cutinase A and Endolase

An Indigo solution was prepared by washing denim with model
10 washing agent. The compositions of model washing agent are as follows:

Sodium dihydrogen phosphate: 6.2g/20L

Sodium citrate: 5.8g/20L

Novasol P: 2.4g/20L

15 Carezyme 1000L (available from Novozymes A/S): 2.8g/20L

The washing conditions were as follows:

Temperature: 55°C

Washing Time: 120 min

20 Enzyme: Model washing agent

Enzyme dosage: 1g/L

Washing liquor: Deionized water (3°dH) / 20L

Denim: Kurabo KD511

Bath ratio: 1:20

25 Washing machine: Wascator (FOM71MP-Lab.)

Swatches (10cm x 10cm) of polyester and polyester/cotton was washed with the indigo solution (pH=6.5) prepared above with Cutinase A and a cellulase (Denimax® 362S), respectively. The
30 conditions were:

Temperature: 55°C

Washing Time: 60min

Washing liquor: Indigo solution (pH=6.5)

Enzymes: Cutinase A and Endolase (Novozym® 613, 3090ECU/g)

35 Enzyme dosage:

0, 1, 3, 5, 10mg enzyme protein/L

Swatch: Polyester, Polyester/Cotton

Swatch size: 10cm X 10cm

Bath ratio: (Polyester X 2, Polyester/Cotton X 2)/L

5 T-O-M: 120rpm

Results:

10 Table 1. Comparison of anti-back staining effect between Cutinase A and Endolase (L* value)

Enzyme	Textile	0mg/L* 1	1mg/L* 1	3mg/L* 1	5mg/L* 1	10mg/L* ¹
Cutinase A	Polyester	68.4	71.6	75.7	79.6	84.0 +/-
		+/-	+/-	+/-	+/-	0.2
		0.8	0.2	0.2	0.4	
	Polyester/Cotton	71.8	71.8	73.9	74.5	76.9 +/-
		+/-	+/-	+/-	+/-	0.2
		0.1	0.1	0.1	0.1	
Endolase	Polyester	68.4	69.6	68.8	69.3	68.6 +/-
		+/-	+/-	+/-	+/-	0.4
		0.8	0.3	0.5	0.4	
	Polyester/Cotton	71.8	72.4	72.7	72.1	72.7 +/-
		+/-	+/-	+/-	+/-	0.4
		0.1	0.2	0.2	0.1	

*¹ Enzyme protein base

The above results show a significant anti-back staining
 15 effect on polyester and polyester/cotton of the cutinase compared with the cellulase. The cellulase did not show any anti-back staining effect on the fabric swatches.

EXAMPLE 2Anti-back staining effect of Cutinase A and B and Lipolase.

5 An Indigo solution was prepared by washing denim with Denimax® 362S in deionised water. The conditions were as follows:

Temperature: 55°C

Washing Time: 120min

10 Enzyme: Denimax® 362S

Enzyme dosage: 1g/L

Washing liquor: Deionized water (3°dH) / 20L

Denim: Kurabo KD511

Bath ratio: 1:20

15 Washing machine: Wasicator (FOM71MP-Lab)

Swatches (10cm x 10cm) of polyester and polyester/cotton was washed with the indigo solution (pH=6.5) prepared above with the cutinases and Lipolase® 100L (available from Novozymes
20 A/S), respectively. The conditions were:

Temperature: 55°C

Washing Time: 60min

Washing liquor: Indigo solution (pH=6.5)

Enzymes: Cutinase A and B and Lipolase® 100L, type EX

25 Enzyme dosage: 0, 10, 30, 50 mg enzyme protein/L (Table 2) and 0, 1, 3, 5mg enzyme protein/L (Table 3)

Swatch: Polyester and Polyester/Cotton

Swatch size: 10cm X 10cm

Bath ratio: (Polyester X 2, Polyester/Cotton X 2) /L

30 T-O-M: 120rpm

Results:**Table 2. Anti-back staining effect of enzymes on polyester and polyester/cotton (L* value)**

Textile	Enzyme	0mg/L* ¹	10mg/L* ¹	30mg/L* ¹	50mg/L* ¹
Polyester	Cutinase A	65.4 +/- 0.3	84.6 +/- 0.1	89.0 +/- 0.0	89.7 +/- 0.1
	Cutinase B	65.7 +/- 0.2	88.3 +/- 0.1	90.3 +/- 0.2	90.8 +/- 0.2
	Lipolase	65.1 +/- 0.2	66.0 +/- 0.4	68.1 +/- 0.2	69.0 +/- 0.4
Polyester/Cotton	Cutinase A	67.9 +/- 0.3	76.3 +/- 0.2	83.3 +/- 0.0	84.6 +/- 0.1
	Cutinase B	68.6 +/- 0.1	82.3 +/- 0.1	86.7 +/- 0.1	86.9 +/- 0.1
	Lipolase	68.1 +/- 0.3	69.2 +/- 0.1	71.8 +/- 0.1	73.6 +/- 0.2

5 *¹ Enzyme protein base

Table 3. Anti-back staining effect of cutinase with low enzyme dosage (L* value)

Textile	Enzyme	0mg/L* ²	1mg/L* ²	3mg/L* ²	5mg/L* ²
Polyester	Cutinase A	63.7 +/- 0.1	68.6 +/- 0.5	75.9 +/- 0.6	81.2 +/- 0.4
	Cutinase B	63.9 +/- 0.2	71.0 +/- 0.1	76.3 +/- 0.3	79.3 +/- 0.6
Polyester/Cotton	Cutinase A	64.2 +/- 0.1	65.5 +/- 0.2	67.5 +/- 0.4	71.0 +/- 0.1
	Cutinase B	64.5 +/- 0.2	66.3 +/- 0.2	69.7 +/- 0.4	72.2 +/- 0.3

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The above results show an anti-back staining effect on polyester and polyester/cotton of the cutinases and the Lipolase.

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EXAMPLE 3Anti-back staining effect of cutinase and lipases at acid pH condition

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An Indigo solution was prepared by washing denim with Cellusoft® L in deionised water. The conditions were as follows:

Temperature: 55°C

10 Washing Time: 120min

Enzyme: Cellusoft® L

Enzyme dosage: 1g/L

Buffer: 1M Acetate buffer (pH=4.8) / 100ml/20L

Washing liquor: Deionized water (3°dH) / 20L

15 Denim: Kurabo KD511

Bath ratio: 1:20

Washing machine: Wasicator (FOM71MP-Lab.)

Swatches (10cm x 10cm) of polyester and polyester/cotton was
20 washed with the indigo solution (pH=5) prepared above with Cutinase A and B, Lipolase® and Lipolase™ Ultra, respectively. The conditions were:

Temperature: 55°C

Washing Time: 60min

25 Washing liquor: Indigo solution (pH=5)

Enzymes: Cutinase A and B, Lipolase® and Lipolase™ Ultra

Enzyme dosage: 0, 10, 30, 50 and 100mg enzyme protein/L (Table 4) and 0, 10 and 30 mg enzyme protein/L (Table 5)

Swatch: Polyester, Polyester/Cotton

30 Swatch size: 10cm X 10cm

Bath ratio: (Polyester X 2, Polyester/Cotton X 2)/L

T-O-M: 120rpm

Results:

Table 4. Anti-back staining effect of Cutinase A and B (L* value)

Enzyme	Textile	0mg/L * ¹	10mg/L * ¹	30mg/L * ¹	50mg/L * ¹	100mg/L * ¹
Cutinase A	Polyester	68.8+/-0.5	74.7+/-1.0	79.6+/-0.2	79.9+/-0.1	82.2+/-1.6
	Polyester/Cotton	65.3+/-0.9	66.8+/-0.2	68.1+/-0.6	67.5+/-1.0	69.9+/-0.6
Cutinase B	Polyester	66.7+/-0.3	80.3+/-0.2	82.4+/-0.6	82.7+/-0.2	81.7+/-0.6
	Polyester/Cotton	67.4+/-0.9	69.2+/-0.4	69.2+/-1.0	70.6+/-0.1	70.8+/-0.5

*¹ Enzyme protein

5

Results:

Table 5. Anti-back staining effect of Cutinase A and B, Lipolase® and Lipolase™ Ultra (L* value)

Textile	Enzymes	0mg/L* ¹	10mg/L* ¹	30mg/L* ¹
Polyester	Cutinase A	68.5+/-0.4	77.9+/-0.5	81.9+/-0.5
	Cutinase B	Ditto	79.6+/-0.9	81.5+/-0.3
	Lipolase®	Ditto	80.0+/-0.8	81.5+/-0.9
	Lipolase™ Ultra	Ditto	75.5+/-0.4	75.6+/-0.4
Polyester/Cotton	Cutinase A	62.0+/-0.8	62.6+/-0.6	63.7+/-0.6
	Cutinase B	Ditto	63.8+/-	64.3+/-

			0.5	0.7
	Lipolase®	Ditto	63.5+/- 0.5	67.3+/- 0.2
	Lipolase™ Ultra	Ditto	63.2+/- 0.5	65.2+/- 0.6

The above results show an anti-back staining effect of the cutinases and the lipases on polyester and polyester/cotton at acidic pH.

CLAIMS

1. A method for reducing the backstaining of fabric or
5 textile, comprising contacting the fabric or textile with a
composition comprising an effective amount of a lipolytic enzyme
(EC 3.1.1).
2. The method according to claim 1, wherein the enzyme is
10 a biopolyester hydrolytic enzyme.
3. The method according to claim 2, wherein the enzyme is
a cutinase
(EC 3.1.1.74), preferably derived from a strain of *Humicola*
15 *insolens*.
4. The method according to claim 1, wherein the fabric or
textile is made of hydrophobic fibres.
- 20 5. The method according to any of the preceding claims, wherein
the fabric or textile is polyester or polyester/cotton,
preferably polyester or polyester/cotton parts of indigo dyed
denim.
- 25 6. The method according to any of the preceding claims, wherein
the amount of enzyme is 1-100 mg of enzyme protein per l of
composition.
7. The method according to any of claims 1-5, wherein the pH is
30 4.5-7.5 and the temperature is 40-60 °C.

8. The method according to any preceding claims for simultaneous reducing the backstaining and formation of localized colour variation, wherein the composition further contains a cellulase and/or pumice.

5

9. A stonewashing composition comprising a lipolytic enzyme and a cellulase.

10. The composition according to claim 9 further comprising a surfactant.

11. The composition according to claims 9-10, wherein the lipolytic enzyme is a biopolyester hydrolytic enzyme, preferably a cutinase (EC 3.1.1.74).

15

12. Use of a composition according to claims 9-11 for reducing backstaining of fabric or textile

20

INTERNATIONAL SEARCH REPORT

In International Application No
PCT/DK 01/00384

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C11D3/386 D06M16/00 D06P5/15

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C11D D06M D06P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 04160 A (NOVONORDISK AS) 6 February 1997 (1997-02-06) claims; example 2	1,4-10
A	WO 99 51808 A (NOVONORDISK AS) 14 October 1999 (1999-10-14) claims 1,9-11,33	1,4-10
A	US 5 912 157 A (VON DER OSTEN CLAUS ET AL) 15 June 1999 (1999-06-15) claims	1,5-10
A	WO 94 07983 A (GENENCOR INT ; CLARKSON KATHLEEN A (US); LARENAS EDMUND (US); WEISS) 14 April 1994 (1994-04-14) claims	1,5-10

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentislan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 851 epo nl,
Fax: (+31-70) 340-3010

Authorized officer

Grittern, A

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No
PCT/DK 01/00384

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9704160 A	06-02-1997	AU 6513096 A	18-02-1997
		EP 0839224 A	06-05-1998
		US 6077316 A	20-06-2000
WO 9951808 A	14-10-1999	AU 3026199 A	25-10-1999
		EP 1066422 A	10-01-2001
		US 6146428 A	14-11-2000
US 5912157 A	15-06-1999	AU 1890095 A	25-09-1995
		WO 9524471 A	14-09-1995
		EP 0749473 A	27-12-1996
		US 6117664 A	12-09-2000
WO 9407983 A	14-04-1994	US 5650322 A	22-07-1997
		AU 4924793 A	26-04-1994
		BR 9307136 A	30-03-1999
		CA 2145873 A	14-04-1994
		EP 0663004 A	19-07-1995
		JP 8502101 T	05-03-1996
		MX 9305945 A	29-07-1994